Final Report on

BIOCHEMICAL ACTIVITIES OF TERRESTRIAL MICROORGANISMS IN SIMULATED PLANETARY ENVIRONMENTS.

NASA Grant NsG 126-61

Series No. 7
Issue No. 12

INTRODUCTION:

The major effort of the research program was directed toward environmental conditions that might serve as constraints upon the evolution or development of microorganisms on the surface of anaerobic planets such as Mars.

STUDIES ON LOW TEMPERATURE FREEZING AND THAWING OF MICROORGANISMS:

Our previous studies on low temperature as a constraint upon microorganisms in simulated Martian environments provided evidence for the ubiquity of terrestrial microbes capable of surviving repeated freezing and thawing (1). In an extension of this line of investigation, we have examined several physiological factors affecting the rate of killing of microorganisms subjected to dry ice temperatures (-78°C.), and thawed to 11°C..

Repeated freezing and thawing of cultures of Escherichia coli in liquid medium, resulted in a linear decrease in the number of surviving cells as a function of the number of freeze-thaw cycles. The slope of this curve provides an index of susceptibility of a culture to death by freezing and thawing. The killing rate was found to be independent of the phase of growth, the state of anaerobiosis during growth, and cell concentration during freezing and thawing. However, the presence of spent growth medium (a filtrate of a dense culture) during freezing protected against cell death. The protective factor(s) is presumably produced during exponential growth, and is heat labile in the presence of alkali. Protection appears to be a consequence of an alteration of the ionic composition of the medium rather than the production of an organic compound. A paper based on the results of freeze-thawing experiments has been accepted for publication (2).

STUDIES ON ALTERNATIVES FOR ATMOSPHERIC OXYGEN:

The absence of detectible quantities of free oxygen in the contemporary atmospheres of other planets in our solar system has raised the question of whether anaerobiosis is a potential constraint upon biochemical evolution. It has been suggested that the absence of molecular oxygen would impose serious restrictions upon oxidative processes in the synthesis and degradation of biologically important compounds (3). By analogy with terrestrial anaerobic environments, a number of inorganic or photochemical oxidants may satisfy the requirements for molecular oxygen as electron acceptors under the anaerobic conditions of planets such as Mars.

Since the microbiological oxidation of the benzene ring requires the addition of molecular oxygen as a prelude to enzymatic cleavage of the aromatic C-C bond, this system provides a convenient model for investigating the participation of alternative electron acceptors in anaerobic degradation reactions. Experiments were designed to determine if nitrate, sulfate or other inorganic oxidants can satisfy the requirement for molecular oxygen in the microbial oxidation of aromatic compounds. With denitrifying bacteria from our culture collection, as well as from enrichment experiments, evidence was obtained for the reduction of nitrate to nitrite during the metabolism of benzoic acid. Similarly, reduction of sulfate to sulfide was observed in analogous experiments with other aromatic compounds. As further evidence of the ability of sulfate reducing bacteria to rupture the aromatic ring, acetic acid was found to be a major degradation product under anaerobic conditions. With vanadate as oxidant, evidence for reduction during substrate oxidation was obtained by colorimetric methods.

Studies on the photochemical oxidation reactions in non-sulfur purple bacteria provide additional support for the hypothesis that a light generated oxidant can substitute for molecular oxygen. In the dark, facultative aerobic photosynthetic bacteria such as Rhodopseudomonas palustris consume oxygen; if they are exposed to light, oxygen uptake is strongly inhibited. The action spectrum for this light induced inhibition of respiration indicates a close correspondence between the inhibitory effect and the spectral absorption of photosynthetic pigments (4). This intimate coupling between photosynthesis and respiration suggests that light induced changes in respiration provide a simple measure of the photochemical oxidant. When respiratory changes were followed polarographically and manometrically, preferential utilization of the photochemical oxidant for substrate metabolism was observed. Addition of 3-(3,4-dichlorophenyl)--1,1-dimethylurea (DCMU) did not appreciably alter the light induced inhibition. The insensitivity to DCMU provides evidence that the photochemical oxidant is independent of oxygen evolution. These results are consistent with the idea that a high potential oxidant can be generated by a light dependent reaction, that the oxidant can compete with respiration, and satisfy the requirement for molecular oxygen.

To obtain evidence for the involvement of anaerobic oxidants in oxidative biosyntheses, we have initiated a study of oxygen-requiring steps in the formation of carotenoids, porphyrins and related photosynthetic pigments. A number of naturally occurring carotenoids have been reported to contain keto, hydroxy, methoxy, and epoxy groups (5).

In each case, a light dependent reaction appears to satisfy the requirement for molecular oxygen to introduce these functions. Studies with photosynthetic bacteria clearly demonstrate that in the absence of light, oxygen is required for such synthetic reactions.

In porphyrin synthesis, conversion of coproporphyrinogen III to protoporphyrin IX requires molecular oxygen for the oxidative decarboxylation reaction (6). However, the presence of such porphyrins in strictly anaerobic photosynthetic bacteria suggests that an oxidant generated by a photochemical reaction can fill the role of molecular oxygen. Since both sulfate reducing bacteria and denitrifiers can synthesize these pigments under anaerobic conditions, the photochemical oxidant may correspond in function to an inorganic electron acceptor. In the absence of such inorganic electron acceptors, the photochemical oxidant must be derived from water.

The emergence of photochemical mechanisms capable of participating in oxidative reactions in anaerobic environments has important implications for biochemical evolution. These were recently discussed at a symposium on photobiology and photochemistry in space research (7).

STUDIES ON ULTRAVIOLET RADIATION AS AN ENVIRONMENTAL CONSTRAINT:

It has been postulated that the ultraviolet (UV) radiation which penetrates to the surface of anaerobic planets such as Mars, would impose a serious constraint upon the development of photobiological processes, and that photosynthetic organisms on Mars, if any, must be able to contend with the UV component which accompanies the visible light. In our earlier studies we observed that UV opaque materials can provide shielding for sensitive genetic determinants (8). From experiments on

In each case, a light dependent reaction appears to satisfy the requirement for molecular oxygen to introduce these functions. Studies with photosynthetic bacteria clearly demonstrate that in the absence of light, oxygen is required for such synthetic reactions.

In porphyrin synthesis, conversion of coproporphyrinogen III to protoporphyrin IX requires molecular oxygen for the oxidative decarboxylation reaction (6). However, the presence of such porphyrins in strictly anaerobic photosynthetic bacteria suggests that an oxidant generated by a photochemical reaction can fill the role of molecular oxygen. Since both sulfate reducing bacteria and denitrifiers can synthesize these pigments under anaerobic conditions, the photochemical oxidant may correspond in function to an inorganic electron acceptor. In the absence of such inorganic electron acceptors, the photochemical oxidant must be derived from water.

The emergence of photochemical mechanisms capable of participating in oxidative reactions in anaerobic environments has important implications for biochemical evolution. These were recently discussed at a symposium on photobiology and photochemistry in space research (7).

STUDIES ON ULTRAVIOLET RADIATION AS AN ENVIRONMENTAL CONSTRAINT:

It has been postulated that the ultraviolet (UV) radiation which penetrates to the surface of anaerobic planets such as Mars, would impose a serious constraint upon the development of photobiological processes, and that photosynthetic organisms on Mars, if any, must be able to contend with the UV component which accompanies the visible light. In our earlier studies we observed that UV opaque materials can provide shielding for sensitive genetic determinants (8). From experiments on

REFERENCES:

1. Packer, E., Scher, S. and Sagan, C., Icarus 2:293 (1963).
2. Packer, E., Ingraham, J. and Scher, S., J. Bacteriol. (in press).
3. Vishniac, W., Aerospace Medicine, 31:678 (1960).
4. Fork, D.C. and Goedheer, J.C., Biochim. Biophys. Acta., 79:249 (1964).
5. ~~Goodwin, T.W., Adv. in Enzymol., 21:295 (1959).~~
6. Sano, S. and Granick, S., J. Biol. Chem., 236:1173 (1961).
7. Scher, S., Fourth Intern. Photobiol. Congress. (in press).
8. Scher, S., Packer, E. and Sagan, C., Life Sciences in Space Research II, p. 352, Ed. Florkin, M. and Dollfus, A., N. Holland Publ. Co., Amsterdam. (1964).
9. Scher, S., Ultraviolet radiation as a constraint upon biochemical evolution on anaerobic planets. (submitted for publication).

PUBLICATIONS:

Sagan, L.A. and Scher, S. Evidence for cytoplasmic DNA in Euglena gracilis. J. Protozool. Suppl. 8:8 (1961).

Scher, S., Scher, B. and Hutner, S.H. Notes on the natural history of Rhodopseudomonas palustris. in Symplosium on Marine Microbiology. pp. 580-587. ed. C. Oppenheimer, C.C. Thomas Publ. Co. Springfield, 1962

Scher, S. and Sagan, L. A. Comparative studies of chloroplast replication: Ultraviolet inactivation and photoreactivation of cytoplasmic DNA synthesis in Euglena gracilis. J. Protozool. Suppl. 9:32 (1962).

Packer, E., Scher, S. and Sagan, C. Biological contamination of Mars: II Cold and aridity as constraints on the survival of terrestrial microorganisms in simulated planetary environments. Icarus 2:293-316 (1963).

Scher, S., Packer, E. and Sagan, C. Biological contamination of Mars: I Survival of terrestrial microorganisms in simulated planetary environments. in Life Sciences and Space Research. II 352-356. ed. M. Florkin and Dollfus. North Holland Publ. Co. Amsterdam, 1964.

Scher, S. and Collinge, J.C. Studies on plastid mutagenesis: evidence for a common site of action of spermine and streptomycin. Plant Physiol. Suppl. 39:34 (1964).

Scher, S. Studies on chloroplast replication: evidence for 5-bromouracil incorporation and chloroplast mutation in Euglena gracilis. in Recent Progress in Photobiology. ed. E.J. Bowen. Academic Press, New York (1965).

Scher, S. Planetary photobiology: prospects and problems. in Recent Progress in Photobiology. ed. E.J. Bowen. Academic Press, New York (1965).

Packer, E., Ingraham, J. and Scher, S. Factors affecting the rate of killing of Escherichia coli by repeated freezing and thawing. J. Bacteriol. 89:718-724 (1965).

Scher, S. and Collinge, J.C. Chloroplast replication: evidence for 5-bromouracil incorporation and chloroplast mutation in Euglena gracilis. Nature 205:828-830 (1965).

Scher, S. Studies on the induction of photosynthetically deficientlplastid mutants: repression of streptomycin and thermal mutagenesis in Euglena. Biophysical Society Abstracts 92 (1965).

Scher, S. Studies on the genetic determinants of chloroplasts in Euglena gracilis: sulfanilamide as a selective agent for chlorophyll-less mutants. in Progress in Protozoology. Intern. Congr. Series 91. Excerpta Medica Foundation, Amsterdam (1965).

Scher, S. Evidence for the non-essentiality of translation errors (misreading) in mutagenesis by aminoglycoside antibiotics. Biochem. Biophys. Research. Commun. (in press)

Other reports and papers prepared for publication:

Sagan, C. and Giver, L. Microwave radiative tranfer in the atmosphere and clouds of Venus. American Geophysical Union Symposium on radiative emmision and thermal structure of the Venus atmosphere. (1961) Interim Report SSLUCB (1961).

Sagan, C. Interstellar panspermia. Symposium on extraterrestrial biochemistry and biology, AAAS. (1961). Interim Report SSLUCB (1961).

Sagan, C. On the nature of the Jovian red spot. Eleventh International Astrophysical Colloquium proceddings. (1962).

Lederberg, J. and Sagan, C. Microenvironments for life on Mars. Proc. Nat. Acad. Sci. 48: 1473-1475 (1962).

Sagan, C. Structure of the lower atmosphere of Venus. Icarus 1:151-169 (1962)

Sagan, C. Direct contact among galactiv civilizations by relativistic interstellar spaceflight. Planetary and Space Science 11:485-498 (1963)

Sagan, C. Biological exploration of Mars. Advan. Astron. Sci. 15:571-581 (1963).

Horowitz, J. and Sagan, C. A study of the published literature on the visual coloration of Mars.

Horowitz, J. and Sagan, C. A model of the Martian blue haze.

Scher, S. Ultraviolet radiation as a constraint upon biochemical evolution on anaerobic planets.

Scher, S. A life detection system based on the photoreversibility principle.

Scher, S. Transfer of biological information between planets. in Evolution and Exobiology, eds. S. Scher and C.R. Weston.

Scher, S. Turnover of biologically important compounds in planetary environments lacking molecular oxygen: the problem of aromatic ring cleavage.

Scher, S. Microbiological oxidation reactions in anaerobic environments: water as a source of oxygen.

Scher, S. and McCalla, D.R. Selection of chlorophyll-less mutants of Euglena gracilis with sulfanilamide.